Implementation of National Institute for Health and Care Excellence (NICE) guidance to measure IgA with all coeliac screens: can an affordable solution be devised?

Short title: IgA measurement in coeliac serological screening

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Summary

There has been a dramatic increase in requests for coeliac disease (CD) serological screening using IgA tissue transglutaminase antibodies (IgA-tTG). Recently, the UK National Institute for Health and Care Excellence has revised its guidance, recommending that total IgA should also be measured in all samples. This is justified since false negative results may occur with IgA deficiency. However, implementation of this guidance will incur considerable expense. Tests that measure IgA-tTG antibodies can detect IgA deficiency, indicated by low background signal. This provides an opportunity to identify samples containing IgA$\leq$0.2g/L, obviating the need for unselected IgA measurement. We investigated feasibility of this approach in two centres that use the EliA™ Celikey™ assay or QUANTA Lite® ELISA to quantify IgA-tTG antibodies. In both cases, total IgA correlated strongly with background IgA-tTG assay signal. Using the Celikey™ assay, a threshold of <17.5 response units achieved 100% sensitivity (95% confidence intervals 79.4% - 100%) for detection of IgA$\leq$0.2g/L, circumventing the need for IgA testing in >99% of sera. A similar principle was demonstrated for the QUANTA Lite® assay, whereby a threshold optical density of <0.0265 also achieved 100% sensitivity (95% confidence intervals 78.2% - 100%) for IgA$\leq$0.2g/L, avoiding unnecessary IgA testing in 67% of cases. These data suggest that CD screening tests can reliably identify samples containing low IgA in a real-life setting, obviating the need for blanket testing. However, this approach requires careful individualized validation, given the divergent efficiency with which assays identify samples containing low IgA.

Introduction

It has been hypothesised that fewer than 20% of patients with coeliac disease (CD) have been identified worldwide [1, 2]. Increasing awareness of this issue has led to a striking increase in IgA tissue transglutaminase (IgA-tTG) antibody test requests [3]. Although rates of diagnosis have improved [4, 5], poorly focussed testing is a recognised problem [3]. To assist clinicians, NICE issued Clinical Guideline 68 (2009) - Recognition and Assessment of Coeliac Disease (https://www.nice.org.uk/guidance; accessed 16-3-2010). However, rates of test positivity remained unchanged and are little better than would be expected from blind screening of the general population [3, 6].

In 2015, NICE guidance was updated to recommend that IgA should be measured in all serum-based CD screening requests [7], aligning with other guidance [5, 8-10]. This is justified on the basis that false negative results are expected with selective IgA deficiency (sIgAD), requiring alternative testing approaches. In sIgAD, IgA is undetectable (<0.07g/L) at age 4 years or above, without secondary cause or abnormality of other immunoglobulin isotypes [15, 16]. Prevalence of sIgAD is 1 in 600 [11], although up to 3% of CD patients may be affected [5, 12-14].

Partial IgA deficiency (pIgAD) is a much commoner occurrence and is characterized by detectable but sub-normal IgA levels [19]. However, pIgAD rarely compromises the performance of the IgA tTG test [14, 20, 21]. To mitigate risk further, the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) recommends that non-IgA based CD screening tests should be used for samples containing IgA≤0.2g/L [8].

A requirement for IgA testing of all CD screens would add significant stress to overburdened health services. One proposed workaround exploits the known ability of IgA-tTG analytical platforms to identify SIgAD [23-29]. Extrapolating from this, we hypothesized that algorithms that robustly identify sera containing IgA$\leq$0.2g/L would obviate the need for blanket IgA measurement. Here, we tested the feasibility of this approach in two centres where distinct CD screening tests are performed.

Materials and Methods

Detection of IgA tTG antibodies

Barnet Hospital receives approximately 10,000 requests for CD screening per annum. Testing was undertaken using the EliA™ Celikey™ assay (Thermo Fisher Scientific, Waltham, MA) run on a Phadia 250 fully automated platform (Thermo Fisher Scientific), as recommended by the manufacturers. In brief, wells are pre-coated with recombinant human tTG. After addition of serum and washing, bound IgA-tTg antibody is detected using beta galactosidase-conjugated anti-IgA secondary antibodies. Next, 4-methylumbelliferyl-β-D-galactoside substrate is added, which yields a fluorescent product. After stopping the reaction with sodium carbonate, fluorescence emission is expressed as response units (RU) which are generated automatically by the software from a six-point calibration curve.

Eastbourne Hospital receives approximately 8,500 requests for CD screening annually. Samples were analysed using the QUANTA Lite® R h-tTG IgA ELISA (Inova Diagnostics, San Diego CA) on a DS2® automated ELISA platform (Dynex Technologies, Worthing, UK) as recommended by the manufacturers. The principle underlying assay performance is similar to the Celikey™ assay, except that the secondary antibody is peroxidase conjugated. After addition of 3,3',5,5' tetramethylbenzidine substrate, a blue product is generated. The reaction is stopped using sulphuric acid which yields a yellow end-point colour that is read at 450nm. Data are expressed as optical density (OD) units which are generated automatically by the software from a five-point calibration curve. In both cases, assay readout (RU or OD

units respectively) is converted to arbitrary units of IgA-tTG antibodies, given that there are no international standards for IgA-tTG antibodies.

Measurement of serum IgA

Serum IgA at Barnet Hospital was measured using an immunoturbidimetric assay run on an Architect ci8200 (Abbott Diagnostics, Lake Forest, IL). At Eastbourne Hospital, the Cobas Tina-quant IgA Gen. 2 assay (Roche Diagnostics, Mannheim, Germany) was used.

Quality assurance and quality control

Both laboratories participate in United Kingdom National External Quality Assessment Service (UK NEQAS) schemes for all assays performed. For each assay, internal quality control was performed throughout using independently sourced third party quality control material.

Statistical analysis

The Shapiro-Wilk normality test was performed using SPSS (version 24) and demonstrated that data were not normally distributed. Consequently, non-parametric statistical testing was used throughout. Kendall's tau b rank correlation was calculated using Wessa, P. (2017), Free Statistics Software, Office for Research Development and Education version 1.1.23-r7, URL http://www.wessa.net/ ; accessed June 25th, 2016 to April 14th, 2017). Median age of patients tested at both sites was compared using the Mann Whitney *U* test, which was performed using SPSS. Receiver operating characteristic (ROC) analysis was performed using Graphpad Prism 6.0g. In all cases,

two-sided *p* values are shown. Ethical approval was not required for this analysis and all data were fully anonymized throughout the analysis. Age related reference ranges for serum IgA were taken from [22].

Results

Detection of low IgA containing samples using the EliA™ Celikey™ assay

The relationship between serum IgA and RU generated using the EliA™ Celikey™ assay was investigated in a data set comprising 367 consecutive and age unselected tests in which RU was below 100 (median age 22 years; Fig. 1a). Strong correlation between these two parameters was observed (Kendall tau b 0.496, p=0). There was also a significant, albeit weak, correlation between RU and age (Kendall tau b 0.19, p=1.2 $x10^{-7}$), in keeping with the age-dependent nature of the normal range for IgA [22].

Next, we explored whether background signal reported by these assays could be used to identify all samples containing IgA≤0.2g/L, a level below which IgA-tTG testing becomes less robust [8]. A receiver operating characteristic (ROC) curve was plotted that employs RU as determined in the EliA™ Celikey™ assay to discriminate between samples containing IgA≤0.2g/L or >0.2g/L (Fig. 1b). Close to perfect test performance is indicated by the area under the curve of 0.99. Using an RU threshold of 17.5, 100% sensitivity (95% confidence intervals 79.4% - 100%) was achieved for all samples containing IgA≤0.2g/L. At these low RU levels, a strong correlation between RU and serum IgA was noted (Kendall tau b 0.95, p=0; Fig. S1). Samples with an RU<17.5 contained IgA≤0.2g/L in 57% of cases (Fig. S1).

Validation of the threshold to detect low IgA using the EliA™ Celikey™ assay

Next, we tested this algorithm using a prospectively collected validation data set comprising 100 consecutive tests in which RU was <50 (Fig. 1c). In this unselected sample (median age 11.5 years; Fig. 1c), a strong correlation was observed once again

between serum IgA and RU (Kendall tau b 0.504; p=0; Fig. 1c). All samples containing IgA≤0.2g/L fell below the threshold RU of 17.5.

Detection of samples containing low IgA using QUANTA Lite® IgA-tTG ELISA

To test applicability of this strategy to an alternative IgA-tTG assay, we undertook a similar analysis using the QUANTA Lite® IgA-tTG ELISA. All sera submitted for 4 randomly selected and sequential CD testing runs (n=264; median age 33 years) were analysed. Correlation between IgA and optical density units was strong (Kendall tau b 0.653 (p=0); Fig. 2a).

ROC curve analysis indicated that a threshold OD of 0.0265 or below allowed the detection of all samples containing IgA≤0.2g/L (95% confidence intervals 78.2% - 100%; Fig. 2b). However, only 15/87 (17.2%) samples with an OD below the threshold contained IgA≤0.2g/L (Fig. S2). Correlation between serum IgA and OD levels below this threshold was also significant (Kendall tau b 0.346; $p = 4.6 \times 10^{-6}$).

Validation of the threshold to detect low IgA using QUANTA Lite® IgA-tTG ELISA

To validate the ability of the QUANTA Lite® ELISA to discriminate IgA≤0.2g/L and >0.2g/L, a consecutive age unselected series of 103 sera in which OD was at or above the threshold (0.0265 – 0.04 OD units), were analysed for IgA. Although correlation between OD units and IgA was weaker (Kendall tau b 0.169; p = 0.014; Fig. 2c), no sample contained IgA ≤0.2g/L (range 0.54g/L – 2.86g/L).

Discussion

This study presents independent audits that investigated whether two IgA-tTG antibody assays could robustly identify sera containing IgA≤0.2g/L. This threshold was selected since ESPGHAN recommends that non-IgA based tests should be used in this setting [8]. Although low levels of IgA may influence RU or OD units in an unpredictable manner (particularly given that it is a rare event), the approach proposed here could eliminate the need for total IgA testing in the majority of cases. Using the Celikey™ assay, most samples with RU<17.5 contained low IgA. Given that <1% of samples tested yield an RU below this threshold, IgA testing could be avoided in over 99% of cases. Similarly, identification of a safe threshold for identifying low IgA levels using the QUANTA Lite® ELISA could obviate the need for measurement of total IgA in 67% of cases.

If test requesting patterns seen at these district general hospitals are extrapolated nationally, it is estimated that 1–1.2 million requests for CD screening are made annually. A serum IgA test has been fully costed at £30 (http://www.thepathlab.co.uk/; accessed 22-2-2017) meaning that implementation of updated NICE guidance would cost £30 million per annum. If our findings are confirmed, virtually all of these funds could be recouped by implementation of a threshold RU on the Celikey™ assay. However, laboratories need to carefully validate and verify such algorithms locally. Alternatively, laboratories may offer combined IgG based testing (eg IgG deamidated gliadin peptide antibodies). These also perform well as CD screening tests when IgA is deficient although this would represent a more costly approach.

Our study has a number of limitations. First, we have demonstrated applicability of this method to two of more than 25 assays available to measure IgA-tTG antibodies. Second, comparison between the performance of these assays is not possible given the different numbers of samples tested, with different age distribution of patients. Third, the possibility of inadvertent ascertainment bias should be considered. Although our analysis was undertaken with unselected serum samples, it is noteworthy that an outlier population in which low RU accompanied higher IgA levels (Celikey™ assay) was more apparent in the training data set compared to the validation set. Ascertainment bias is a well-recognised issue in many studies of CD screening serology [30].

The need for blanket IgA testing of coeliac screens has been questioned since it rarely unmasks a diagnosis of CD and may lead to unnecessary biopsy [31, 32]. It also increases workload and test turnaround time and marginally increases the negative predictive value of the IgA-tTG test (from 99.8% to 99.9%), but at a cost of over $32,605 per false negative test missed [33]. We also encountered issues with confused users seeking advice on how to investigate borderline high or low IgA results that had not been requested in the first instance and which were of dubious clinical significance.

NICE responded to these points, arguing that application of IgA-tTG tests to identify sIgAD represents the incorrect use of these assays [34]. NICE speculated that false positive results might arise due to rheumatoid factor or haemolysis. No evidence was presented in support of these concerns and we did not identify any such issues in these audits. NICE highlighted the potential benefit of detecting immunodeficiency using blanket IgA testing, an incident so rare to warrant reporting [35]. We view this as an inappropriate use of CD screening testing. Blind screening is rarely cost effective and the commonest immunodeficiency that would be detected is sIgAD, for which screening

cannot be justified on the basis of cost-benefit analysis. The updated NICE guideline reverses their previous recommendation to check for IgA deficiency “if the laboratory detects a low or very low optical density on IgA-tTG test”. Consideration should be given to reinstatement of this earlier recommendation. Such an approach can be adapted to maintain consistency with ESPGHAN guidance [8] but must be dependent upon the ability of the laboratory to set a robust threshold that identifies all low IgA samples.

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Conflict of Interest

There are no competing interests.

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Figure Legends

Fig 1. Use of the EliA™ Celikey™ assay to detect sera containing low levels of IgA. (a) A training data set was generated using 367 consecutive CD screening samples with RU<100. Correlation between IgA and RU is shown together with age distribution of patients. The lower end of the IgA reference range for each age cohort is shown in brackets. (b) Receiver operating characteristic curve indicating the selection of the RU threshold for optimum assay performance. (c) To validate this threshold, a data set was generated using a consecutively analysed set of 100 sera in which RU was <50. Correlation between IgA and RU is shown together with age distribution of patients. Mo – months; yr – years.

Fig 2. Use of the QUANTA Lite® ELISA assay to detect sera containing low levels of IgA. (a) Two hundred and sixty four consecutively submitted CD screening samples were analysed for IgA content. Correlation with OD units is shown together with age distribution of patients. The lower end of the IgA reference range for each age cohort is shown in brackets. (b) Receiver operating characteristic curve indicating the selection of the RU threshold for optimum assay performance. (c) To validate this threshold, a data set was generated using a consecutively analysed set of 103 sera in which OD was at or above the optimised threshold (range 0.0265 – 0.04 OD units). Correlation between IgA and RU is shown together with age distribution of patients. Mo – months; yr – years.

Figure 1

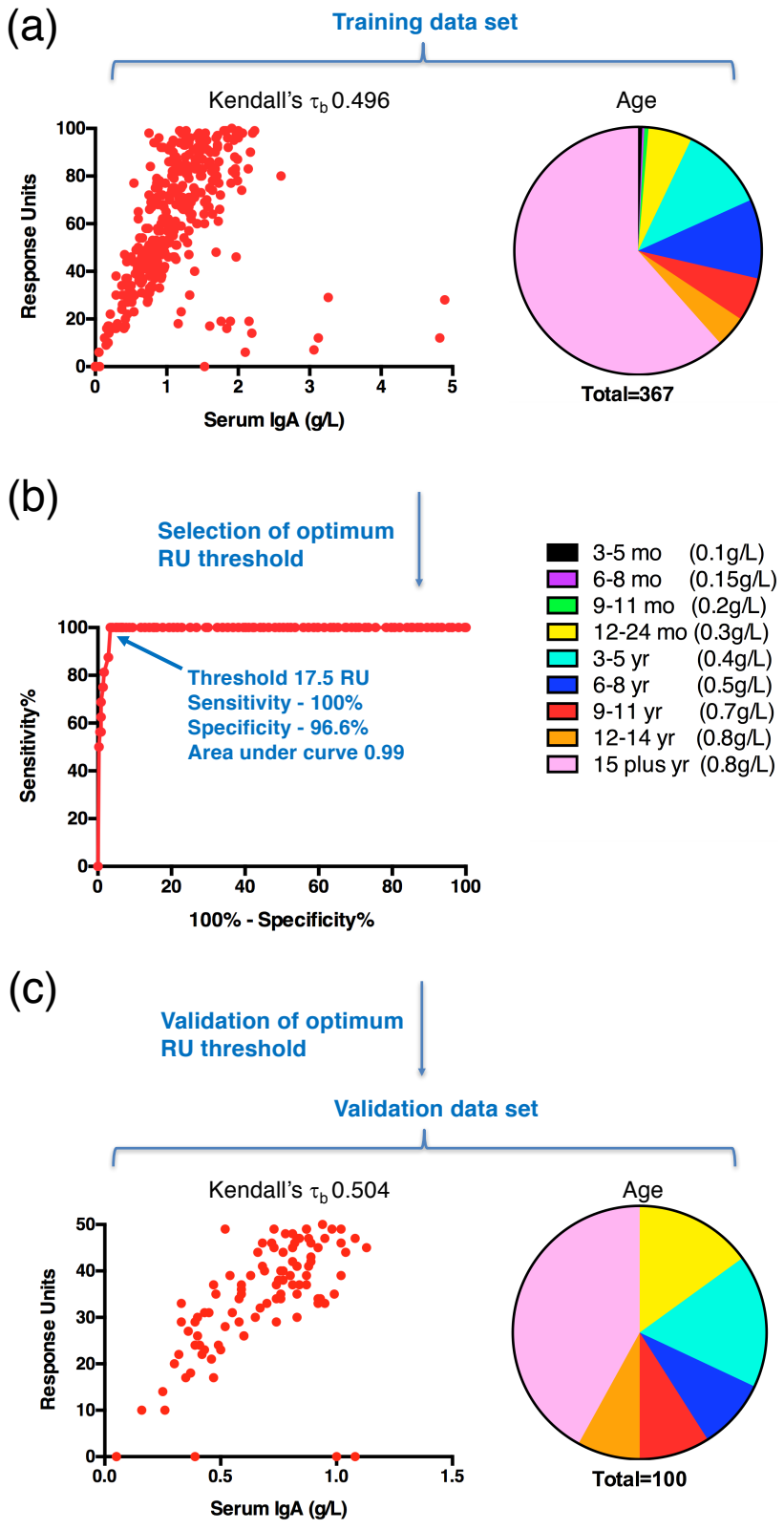
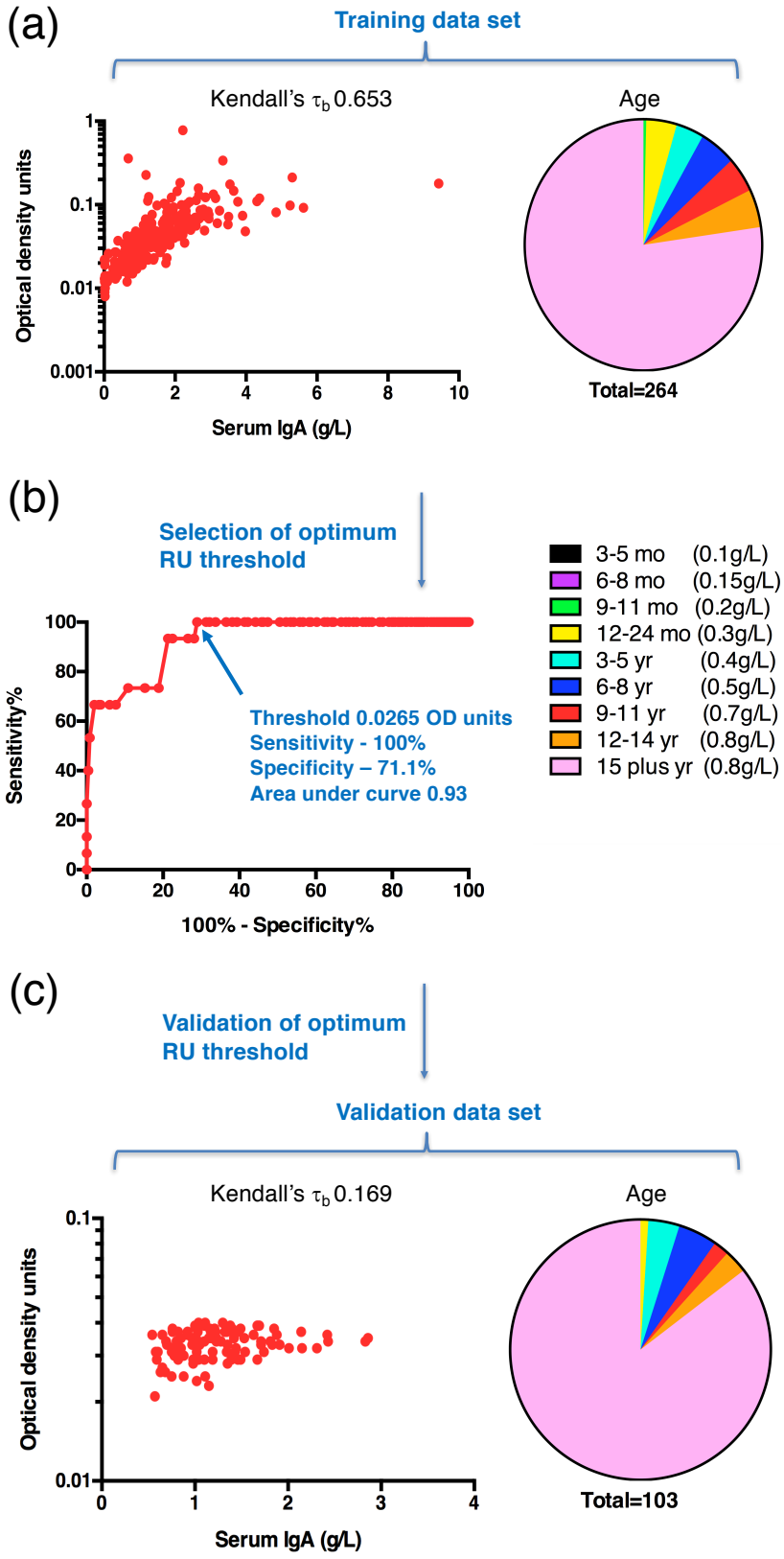

(a)
Training data set
Kendall's τb 0.496
Response Units
Serum IgA (g/L)
Age
Total=367
(b)
Selection of optimum RU threshold
Threshold 17.5 RU
Sensitivity - 100%
Specificity - 96.6%
Area under curve 0.99
Sensitivity%
100% - Specificity%
3-5 mo (0.1g/L)
6-8 mo (0.15g/L)
9-11 mo (0.2g/L)
12-24 mo (0.3g/L)
3-5 yr (0.4g/L)
6-8 yr (0.5g/L)
9-11 yr (0.7g/L)
12-14 yr (0.8g/L)
15 plus yr (0.8g/L)
(c)
Validation of optimum RU threshold
Validation data set
Kendall's τb 0.504
Response Units
Serum IgA (g/L)
Age
Total=100

Figure 2


(a)
Training data set
Kendall's τb 0.653
Optical density units
1
0.1
0.01
0.001
0
2
4
6
8
10
Serum IgA (g/L)
Age
Total=264
(b)
Selection of optimum
RU threshold
Sensitivity%
100
80
60
40
20
0
0
20
40
60
80
100
100% - Specificity%
Threshold 0.0265 OD units
Sensitivity - 100%
Specificity – 71.1%
Area under curve 0.93
3-5 mo (0.1g/L)
6-8 mo (0.15g/L)
9-11 mo (0.2g/L)
12-24 mo (0.3g/L)
3-5 yr (0.4g/L)
6-8 yr (0.5g/L)
9-11 yr (0.7g/L)
12-14 yr (0.8g/L)
15 plus yr (0.8g/L)
(c)
Validation of optimum
RU threshold
Validation data set
Kendall's τb 0.169
Optical density units
0.1
0.01
0
1
2
3
4
Serum IgA (g/L)
Age
Total=103